APPLICATIONS OF NUCLEAR QUADRUPOLE RELAXATION TO THE STUDY OF ION BINDING IN SOLUTION

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In the following a review of some applications of the nuclear quadrupole relaxation method to the study of chemical association will be made. The papers discussed will be denoted by reference numbers 1 - 9 as follows:

1. Lindman, B., Forsén, S. and Forslind, E., Nuclear Quadrupole Relaxation of ^{79}Br in Aqueous Solutions of Quaternary Ammonium Bromides. J. Phys. Chem., 72, 2805 (1968).

2. Lindman, B., Wennerström, H. and Forsén, S., A Bromine-79 Nuclear Magnetic Resonance Study of the Structure of Aqueous Solutions of Mono-, Di- and Trialkylammonium Bromides. J. Phys. Chem., 74, 754 (1970).

3. Lindman, B. and Ekwall, P., Solutions of Alkali Soaps and Water in Fatty Acids. IV.NMR Investigation of the Sodium Ion Binding in Solutions of Sodium Caprylate and Water in Caprylic Acid. Kolloid-Z. Z. Polym., 234, 1115 (1969).

4. Lindblom, G., Lindman, B. and Mandell, L., A Study of Counter-ion Binding to Reversed Micelles by Nuclear Magnetic Quadrupole Relaxation of ^{81}Br. J. Colloid Interface Sci. In press.

5. Zeppezauer, M., Lindman, B., Forsén, S. and Lindqvist, I., A ^{81}Br Nuclear Magnetic Resonance Study of Bromide Ion Binding to Proteins in Aqueous Solution. Biochem. Biophys. Res. Commun., 37, 137 (1969).

6. Csopak, H., Lindman, B. and Lilja, H., ^{81}Br and ^{35}Cl Nuclear Magnetic Resonance Studies of E. Coli Alkaline Phosphatase. Fed. Eur. Biochem. Soc. Lett., 9, 189 (1970).

7. Lindman, B., Zeppezauer, M. and Åkeson, Å., The Use of Chloride Ion as Reporter Group for Changes in Protein Conformation. A ^{35}Cl NMR Study of the Binding of Coenzyme and Inhibitors to Horse Liver Alcohol Dehydrogenase. Proceedings of the Wenner-Gren Symposium on "Structure and Function of Oxidation Reduction Enzymes." Stockholm 1970. In press.

8. Danielsson, I., Lindman, B. and Ödberg, L., NMR Studies on Aqueous Alkanoate Solutions. Suom. Kemistilehti B, 43, 209 (1970).

9. Lindqvist, I. and Lindman, B., Quadrupole Relaxation Studies of Humic Acids. Acta Chem. Scand., 24, 1097 (1970).

In all cases, the nuclear magnetic relaxation of ionic nuclei has been studied. The nuclei mainly investigated are ^{23}Na (3), ^{35}Cl (6, 7), ^{79}Br (1, 2), ^{81}Br (4-6) and ^{85}Rb (8, 9). The articles discussed are chiefly concerned with the following three types of system:

a) Aqueous solutions of alkyl-substituted ammonium bromides (1, 2),

b) Solutions containing association colloids (2-4, 8),

c) Aqueous solutions of naturally occurring macromolecules (5-7, 9).

1. Nuclear Magnetic Resonance in Electrolyte Solutions

During the last decade nuclear magnetic resonance (NMR) has developed into an extremely useful method for the study of electrolyte solutions. Depending on the type of NMR method employed, information may be gained regarding either the interactions present in the system or the micro-dynamic properties of the solution. The former type of information is normally obtained from studies of chemical shifts, whereas dynamic processes may be elucidated from different types of nuclear magnetic relaxation studies. The principles underlying the application of NMR to electrolyte solutions have been thoroughly presented in two recent review articles by Hertz (10) and Devcrell (11). As a background for the discussion some typical types of information obtainable from NMR studies will be briefly pointed out.[x]

The observation that the position of the water proton magnetic resonance (PMR) signal changes when solutes are added to water has been the basis of several investigations of solute-water interactions by NMR (12). In this way, information has been obtained concerning the changes in hydrogen - binding in water that occur on introduction of solutes. Resolution of the observed chemical shifts into ionic contributions showed that the smallest alkali ions are structure-making (i.e. they stabilize the water lattice), while large halide ions are structure-breaking.

In some cases the residence times of the water molecules in the hydration sphere of an ion are so great that more than one water NMR signal is observable. Under such conditions, the average hydration number of the

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[x] For more extensive reference lists see refs. 10 and 11.

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ion may be directly calculated from the integrated intensities of the separate NMR signals. Generally, the ^{17}O magnetic resonance is more useful than PMR, owing to the greater chemical shifts in the case of ^{17}O. By ^{17}O NMR, hydration numbers of Be^{2+}, Al^{3+} and Ga^{3+} have been determined (13), and furthermore estimates of the rate of exchange of water molecules have been made.

Nuclear magnetic relaxation has been demonstrated to be a very powerful method for the elucidation of the microdynamic properties of electrolyte solutions. From a measurement of the longitudinal relaxation time of water protons, deuterons or ^{17}O nuclei, the water reorientation time can usually be quite accurately calculated (10). The reorientation time for water in electrolyte solutions depends strongly on the solute. Thus, small cations (e.g. Li^+, Mg^{2+}) have a marked slowing-down effect on water molecular reorientation (14). Large anions (e.g. I^-, NO_3^-), on the other hand, tend to speed up water rotation (14, 15). Interesting is the fact that alkyl groups drastically reduce the water rotation rate (16, 17).

By a NMR pulse method, self-diffusion coefficients may be absolutely and conveniently measured. The principle of the method depends on the fact that the motion of nuclear spins in an applied magnetic field gradient will measurably affect the transverse nuclear magnetic relaxation. The diffusing species are thus labelled by their nuclear spins, which in many cases is preferable to labelling by radioactive nuclei as in tracer techniques. By this method, the effect of various solutes on the translational motion of the water molecules has been studied extensively. (See e.g. refs. 14 and 18.) Again ions containing alkyl groups are remarkable in their great slowing-down effect on the motion

of the water molecules (19).

As is well known, NMR constitutes an efficient method for the study of the kinetics of fast reactions. In this way, the rate constants for exchange of solvent molecules between the first coordination shell of a paramagnetic ion and the bulk solvent have been determined (11). Similarly, from the relaxation of quadrupolar nuclei, it is possible to determine exchange rates, such as for the exchange of iodide ions between the bulk of the solution and different complexes formed by mercuric and iodide ions (20, 21).

Apart from applications to aqueous solutions of small ions, nuclear magnetic resonance has contributed to the structure elucidation of solutions containing macromolecules or large aggregates formed by amphiphilic molecules. Thus, for example, the interactions between enzymes and small molecules (such as inhibitors and substrates) may conveniently be studied by various NMR methods. (See e.g. refs 22-24.) The tendency of soap molecules to associate and form micelles (25-27), and different types of liquid crystals (28, 29), are other fields where NMR has found extensive use. The binding of small ions in aqueous solutions of macromolecules and in micellar solutions will be treated in some detail below. First, however, the principles underlying the study of ion binding by the nuclear quadrupole relaxation method will be briefly considered.

2. Quadrupolar Relaxation in Electrolyte Solutions

For nuclei such as ^{1}H and ^{19}F, a change in type or degree of binding will lead only to moderate changes in the nuclear magnetic relaxation

rates. On the other hand, e.g. ^{2}H and ^{35}Cl relaxation rates are very sensitive to changes in the binding properties of the corresponding atoms. This difference is due to the fact that ^{2}H and ^{35}Cl nuclei have non-zero electric quadrupole moments. A necessary condition for a nucleus to have an electric quadrupole moment is that the spin quantum number (I) must equal or exceed unity. For nuclei with quadrupole moments, the interaction between the electric quadrupole and fluctuating electric field gradients at the nucleus constitutes, in liquid systems, such an effective relaxation mechanism that other contributions to the relaxation, such as e.g. magnetic dipolar interactions, normally may be neglected.

The theory of nuclear quadrupole relaxation has been formulated by Abragam and Pound (30, 31), who derived the following expression (see ref. 32)

$$\frac{1}{T_1} = \frac{1}{8} \frac{2I+3}{I^2(2I-1)} \left(\frac{eQ}{\hbar}\right)^2 J \qquad (1)$$

Eq. (1) is valid for the case of extreme narrowing, i.e. when the correlation time of the interaction, τ_c, is much smaller than the inverse Larmor precession frequency. In eq. (1), T_1 is the longitudinal relaxation time, eQ the nuclear electric quadrupole moment, e the electronic charge, $\hbar = \frac{h}{2\pi}$ (h is Planck's constant) and J the spectral density. If there exists a motional correlation between the ion studied and the species in the solution setting up the field gradients, eq. (1) may be written (31, 32)

$$\frac{1}{T_1} = \frac{3}{40} \frac{2I+3}{I^2(2I-1)} \left(1 + \frac{\eta^2}{3}\right) \left(\frac{e^2Qq}{\hbar}\right)^2 \tau_c \qquad (2)$$

Here eq is the largest of the principal components of the electric field gradient tensor

at the nucleus, and η the asymmetry parameter. Normally, the assumption of an axially symmetric field gradient is a good approximation, and in this case η may be neglected. In any case, variations in η can only account for a small part of the changes in relaxation rate which were observed in the work underlying this review. Whenever chemical association occurs, the life time of the complex formed is expected to be long compared to the correlation times normally encountered (10^{-9} sec < τ_c < 10^{-12} sec), and hence motional correlation is present. There seems to be no doubt that eq. (2) applies to the typical association problems investigated in refs. 3-9. For the alkylammonium bromide solutions studied in refs. 1 and 2, little is known about association phenomena, and alternative expressions for the relaxation rate should also be considered. The overlap mechanism proposed by Deverell (1, 33) has been very successful in explaining the relaxation of nuclei of the largest ions in aqueous alkali halide solutions, but of course the overlap integrals can not vary with the alkyl chain length to the extent that would be predicted by the data presented in refs. 1 and 2. The Hertz (34) -Valiev (35) expression for the interionic contribution to the nuclear relaxation rate predicts a dependence on the counter-ion size which is opposite to that observed. (Hertz (34) and Valiev (36) also considered the effect of rotatory, translational and vibrational motion of water molecules in the ionic hydration shell on the ionic nuclear relaxation rate.)

According to the arguments presented, it has been assumed that eq. (2) is applicable, and therefore the following discussion will throughout be based on this equation.

It may be enlightening for the following discussion if we begin by exemplifying the changes in relaxation rate occurring in liquid systems. Let us choose some examples from ^{35}Cl NMR at ambient temperature. A "free" aqueous chloride ion (i.e. hydrated, as in a dilute sodium chloride solution) is nearly symmetrically solvated, and thus the nucleus experiences small field gradients. Furthermore, such a chloride ion is involved in rapid motion, which makes the correlation time small. Consequently, the relaxation rate is small, and corresponds to a NMR line-width (cf. below) of about 13 Hz (24). In covalent compounds, the ^{35}Cl nucleus senses field gradients which are an order of magnitude greater, and accordingly the ^{35}Cl NMR line-widths of e.g. $HgCl_4^{2-}$ and CCl_4 are of the order of 10^4 Hz (37, 38). For a compound of the type R-Hg-Cl, the correlation time increases as the size of the R group is increased. Thus, a chlorine coupled to a mercury-protein complex gives a ^{35}Cl line-width which is of the order of 10^6 Hz (37).

From these considerations, it is evident that the quadrupole relaxation rate is extremely sensitive to changes in the binding and in the micro-dynamic properties of the system, and thus it should be possible to conveniently study chemical association phenomena by this method. The great drawback of the method is that for ionic nuclei, a separation of the two factors determining the relaxation rate, i.e. the field gradients and the correlation time, is difficult to accomplish.

3. Some General Remarks

The studies summarized here are all concerned with the determination of nuclear magnetic resonance line-widths. The width of a NMR signal is (by definition) proportional to the transverse relaxation rate, $\frac{1}{T_2}$. In

the case of extreme narrowing, T_1 equals T_2 and consequently for quadrupolar relaxation, equation (3) is valid for a particular nucleus.

$$\Delta B = \frac{1}{\gamma}\Delta\nu = k(eq)^2 \tau_c \qquad (3)$$

In this equation ΔB and $\Delta\nu$ are the NMR line-widths in magnetic field and frequency units respectively, γ is the nuclear magnetogyric ratio, and k is a constant.

Depending on the experimental procedure employed, two different definitions of the line-widths have been used. If the frequency of the magnetic field modulation is very great compared to the line-width, the NMR absorption signal is recorded. In this case, the width at half height of the NMR signal was measured. If, on the other hand, the modulation frequency is much smaller than the line-width, the derivative of the absorption signal is obtained. The line-width was then taken as the distance between the maximum and minimum slope of the absorption curve.

Since no equipment for measuring T_1 was available in our laboratory, the validity of the assumption $T_1 = T_2$ was tested by measuring the line-width at different resonance frequencies. (The equality between T_1 and T_2 has been verified experimentally for some electrolyte solutions by Eisenstadt and Friedman (39).)

When studying association phenomena, one has to consider the possibility of chemical exchange of the nuclei between various sites with different NMR parameters. If the lifetimes in the different sites are much smaller than the corresponding relaxation times, the line-width will simply be a weighted average of the line-widths, ΔB_i, characterizing the different sites (10, 40):

$$\Delta B_{obsd} = \Sigma\, p_i\, \Delta\nu_i \qquad (4)$$

p_i is the probability for the nucleus to be located in site i. We will denote the situation described as "fast exchange". If the exchange is slower, the NMR signals will depend on the residence times of the nuclei in the various sites. The different cases can, as proposed by Hertz (20, 41), be distinguished by studying the temperature dependence of the line-widths, or by comparing the line-widths for two isotopic nuclei with different quadrupole moments. The latter method is, when applicable, advantageous.

After these general considerations we will now turn to a summary of the results for the various experimental studies.

4. Aqueous Solutions of Alkyl-substituted Ammonium Bromides

Aqueous solutions of alkylammonium salts have, especially in the last few years, been studied extensively by various methods.(A review has been given by Kay (42).) The most important reason for the great interest concerning the structure of these solutions, is that they are model systems for the study of the interaction between water and nonpolar groups. Of course, the high solubilities of these salts in water have contributed to the choice of these solutions as a model.

Despite the fact that many investigations have been concerned with the structure of these solutions, the amount of definite information accumulated is limited. On one point, however, there is general agreement by most workers in the field, viz. that the alkyl groups in

the cations have a structure-forming effect on the water lattice (43). Thus, NMR studies show that both the rotational and the translational motions of the water molecules are slowed down when tetraalkylammonium halides are dissolved in water (16, 17, 19, 44). Also, near-infrared studies (45), dielectric studies (46) and viscosity studies (47) have provided evidence for a structure-making effect of the large cations. Another pertinent observation is the formation of stable hydrates, where the cations are enclosed in water "cages" (48, 49). An interesting feature of these hydrates is that the anions replace water molecules in the water "clathrate" structure (49).

During studies of the ^{79}Br resonance in aqueous solutions of tetraalkylammonium bromides, it was observed that these cations strongly broaden the ^{79}Br NMR signal (1). The broadening effect may be orders of magnitude larger than that of other univalent cations such as alkali ions. Later, a study of aqueous solutions of mono-, di- and trialkylammonium bromides was performed (2). The most important observations are the following:

1. The relaxation rate of ^{79}Br increases strongly with increasing length of the alkyl chain, and with increasing number of alkyl chains.

2. The relaxation rate increases, at moderate concentrations, nearly linearly with salt concentration.

3. The line-widths vary more strongly with temperature than do the line-widths of aqueous alkali halide solutions. The apparent energy of activation of the relaxation process increases with increasing substitution on the nitrogen.

4. The exchange between different binding sites proceeds in a time short compared to the relaxation times.

5. In nonaqueous solvents, the line-width may be practically independent of cation size, or show a reverse dependence on cation size compared to aqueous solutions.

From an as yet unpublished investigation (50), the following observations may be added:

6. For the corresponding chlorides and iodides the same effects (from ^{35}Cl and ^{127}I NMR) are observed. The magnitude of the relative line-broadening (i.e. the observed line-width divided by that of a "free" ion) varies only slightly between the three ions.

7. Substitution of nitrogen for phosphorus in tetrabutylammonium bromide does not significantly affect the relaxation rate.

8. If the groups on the nitrogen are made more polar, this tends to reduce the line-width.

It is apparent that since these cations strongly influence the anionic nuclear magnetic relaxation, there must be some (direct or indirect) type of anion-cation interaction. Formation of contact ion-pairs is excluded, from the observations quoted. Thus, contact ion-pairing is expected to increase with decreasing ionic size, which has also been demonstrated to be the case in some non-aqueous solvents (51), while we observe an increased interaction with increasing ion size. The measurements in different solvents indicate that the effect is related to the specific structural properties of water. On the basis of our measurements, we have found that the species in the solution responsible for the very

effective bromine relaxation are not contact ion-pairs but rather solvent-separated ion-pairs. The concept of water structure-enforced ion-pairing introduced by Diamond (52) seems to be a good characterization of the situation. More accurately, we may describe the structure of these solutions as follows: The alkyl groups of the cations will tend to stabilize the water lattice in their neighbourhood. The amount of structure-modified water will increase with the size of the cations. The anions are incorporated in this structure-stabilized water lattice.

Since the correlation times for the water molecules in the vicinity of the cations are, at least approximately, known from proton and deuteron T_1 measurements (16) it could be demonstrated that changes in the correlation time can account for only a minor part of the observed line-broadening effect. It was hence concluded that the bromine nuclei in the vicinity of the cations sense greater field gradients than in an aqueous alkali halide solution. The great field gradients can arise either from a strengthened anion-water coupling near the bulky cations, or from unsymmetrical hydration in the vicinity of the cation. There is no conclusive evidence for either interpretation, but the very high activation energies observed for the relaxation process make the former explanation more likely to be the correct one. These questions are discussed more thoroughly in ref. 50.

Other workers have discussed their data in terms of contact ion-pairing (53), and even micelle formation (53, 54). Contact ion-pairing is, as argued above, not compatible with our findings. Furthermore, the partial molal volumes which were ascribed to ion-pairing by Wirth (53) have by other workers (55-57) been interpreted as being due to modifications of the water structure by the tetraalkylammonium ions. The concept of

Wirth (53) to assume that increased structuring of water does not occur in these solutions, which is at variance with other studies (see above). Recently, ^{14}N NMR relaxation data have been presented which support our view that contact ion-pairing is not important in these aqueous solutions (58). Tetraalkylammonium ions have a structure quite different from molecules or ions known to aggregate into micelles (cf. next section), and therefore micelle formation is not likely to occur. However, on the basis of solubilization and volume data, Wirth (53, 54) postulates micelle formation for the larger tetraalkylammonium ions. Recent measurements by ^{81}Br NMR show that the usual drastic increase (cf. below) in counter-ion NMR relaxation rate does not occur at the critical micelle concentration in this case (50). Light-scattering measurements (59) have also failed to reveal any cation-cation association in the region just above the critical micelle concentration, as proposed by Wirth (53, 54).

5. Solutions Containing Association Colloids

A characteristic property of aqueous surfactant solutions is the very sharp changes in many experimental quantities at a certain concentration (60). This is due to the formation of large aggregates, micelles. The structure of micelles in aqueous solution is not yet settled, but much evidence favours a spherical model, in which the surfactant molecules are arranged with their hydrophobic groups towards the interior of the sphere, and the hydrophilic groups outwards in contact with water. The aggregation number and the concentration at which micelles first form (critical micelle concentration, c.m.c.) are strongly dependent on the length of the surfactant molecule and on its end group.

It is to be expected that when typical soap molecules aggregate into micelles there will be changes in the various NMR parameters. In this way c.m.c. values have been estimated from soap and water proton chemical shifts (61) and relaxation times (62, 63). A general observation is that the PMR frequency is rather insensitive to micelle formation, and the observed effects are often masked by the uncertainty in the susceptibility correction (cf. ref. 8) (^{19}F NMR chemical shifts, on the other hand, have been used with great success (27, 64).) A large fraction of the counter-ions are, more or less firmly, attached to micelles composed of ionic amphiphiles. Therefore, as first observed by Eriksson et al. (65), the quadrupole relaxation of counter-ion nuclei will start to get considerably more effective at the c.m.c.

In ref. 8, the quadrupole relaxation method was applied to a study of the binding of rubidium ions in some aqueous rubidium alkanoate solutions by ^{85}Rb NMR. It was found that at low concentrations, the ^{85}Rb relaxation rate is constant, but at a concentration which is roughly equal to the c.m.c. (as determined by other methods) the NMR signals begin to broaden. At moderate concentrations, the results are explainable in terms of a model with two different sites for the counter-ions, one representing free Rb^+ ions and the other Rb^+ ions bound to micelles. The exchange between the two sites proceeds faster than the relaxation. The binding of rubidiums ions to the micelles is constant in a rather large concentration range above the c.m.c. (cf. ref. 66). Above this region, the counter-ion binding is enhanced. In this work (8), also the proton chemical shifts and the proton longitudinal relaxation times (in D_2O solutions) were determined for some alkali alkanoate solutions. As mentioned above, structural information from PMR chemical shifts is not readily obtained (cf. also ref. 67). The plots of $1/T_1$ vs. concentration

show an increased slope after the c.m.c. The effect is more pronounced for sodium caproate than for the lower homologues. The increase in relaxation rate at the c.m.c. is due to two effects: Firstly, the interaction term increases as the inter-proton distances decrease, and secondly the soap molecules in a micelle move more slowly than in a molecule-disperse solution.

If one considers the ratio $\Delta B_{obsd}/\Delta B_o$, where ΔB_{obsd} is the observed counter-ion NMR line-width, and ΔB_o the line-width of the ionic nucleus in question in water at infinite dilution, a marked difference between cations (Na^+, Rb^+) and anions (Br^-) is observed (2, 8, 65, 66, 68-70). For ^{85}Rb in aqueous hexanoate and octanoate solutions, this quantity does not exceed 3, whereas in alkylammonium bromide solutions it is an order of magnitude greater. We may from these data conclude that the change in binding properties on going from an alkali halide solution to a micellar solution is much smaller for Rb^+ than for Br^-. This indicates that the hydration of rubidium ions is preserved to a high degree when the ion is bound to a micellar surface. The difference between Rb^+ ions and Br^- ions in this respect is also evident when $\Delta B_{mic} / \Delta B_o$ or the energies of activation of the relaxation process are considered (2, 66, 70). (ΔB_{mic} is the line-width characterizing the micellarly bound counter-ions.) Both these quantities are considerably greater for bromide ions than for rubidium ions.

In ternary systems composed of water, an association colloid and a second amphiphile such as an alcohol or a fatty acid, two different regions with isotropic solutions exist. One is rich in water (L_1 phase), and the other is rich in the alcohol or fatty acid (L_2 phase). Over a large range of concentrations, the L_2 solutions contain micelles of the reversed type, i.e. micelles with a water core which is surrounded by the

hydrophilic groups of the amphiphilic molecules (71). In this treatment, counter-ion quadrupole relaxation measurements in the L_2 solution phases in the ternary systems cetyltrimethylammonium bromide (CTAB) - hexanol - water and sodium caprylate (NaC_8) - caprylic acid - water will be discussed. The phase diagrams have been determined by Ekwall and co-workers (72, 73).

In the L_2 solutions in the CTAB - hexanol - water system, the bromide ion binding was studied by ^{81}Br NMR (4). In a large region of the L_2 phase at high water concentrations, the quadrupole relaxation rate is almost independent of the composition of the solution. Since the line-widths in this area are essentially the same as in concentrated aqueous CTAB solutions, it could be assumed that the bromide ions are located in the water cores of the reversed micelles. If the water content in the micellar solutions is diminished at a constant ratio of CTAB to hexanol, the line-widths start to increase when the molar ratio of water to CTAB is about 15. These data indicate that athigh water contents the bromide ions are extensively hydrated, but that at low water contents some of the counter-ions are rather firmly attached to the micellar surfaces. By varying the hexanol content at a constant ratio of CTAB to water, it was shown that on going from the micellar region to the region of molecule-disperse solutions a dramatic rise in counter-ion relaxation rate results and presumably reflects an extensive ion-pairing at high hexanol contents. In the lamellar liquid crystalline phase in this system, the ^{81}Br relaxation rates are somewhat larger, but the changes in bromide ion binding on going from an isotropic solution to a mesomorphous phase are small (74).

In solutions of sodium caprylate and water in caprylic acid, the ^{23}Na NMR line-width is primarily determined by the water content (3). On the addition of water to a solution of sodium caprylate in caprylic acid, the ^{23}Na line-width decreases, at first very rapidly, and then, at higher water concentrations, much more slowly. In water-free solutions of NaC_8 in caprylic acid, the ^{23}Na relaxation rate is very fast, which was assumed to be due to ion-pairing. According to other investigations (75, 76), the anionic complexes formed are composed of one caprylate ion and two caprylic acid molecules. On the addition of water, the sodium ion binding to these complexes is loosened due to hydration. At higher water contents, micelles form in this solution phase (71). In an intermediate water concentration range, the micelles are of the reversed type, but at the highest water contents, normal micelles in an aqueous environment exist (71). The rather small ^{23}Na NMR line-widths in the micellar solutions indicate that the sodium ions are hydrated to a great extent. Furthermore, in a study of the L_2 solutions in the system sodium caprylate - decanol - water, it was observed that hydration of the sodium ions is the most important factor in the determination of the ^{23}Na NMR line-width (68).

Another noteworthy observation in these studies (3, 4) was that, contrary to the simple Debye treatment (77), the viscosity and the NMR line-widths often show an entirely opposite concentration dependence. The explanation of this phenomenon is that as reversed micelles form, the viscosity increases strongly, while the counter-ion quadrupole relaxation rates decrease due to the incorporation of the counter-ions in the water cores. When this phenomenon is observed, it is a good indication of extensive association in the solutions.

6. Ion Binding to Macromolecules in Aqueous Solutions

The usefulness of NMR in the study of protein structure and function is emphasized by an enormous number of applications in the last few years (78). Steady-state studies have been concerned with the protein itself, or with inhibitors or substrates. So-called relaxation enhancement studies have provided enlightenment into the function of enzymes in a number of cases (22). Water relaxation studies in protein solutions have demonstrated that there is on the average a slowing down in the motion of the solvent molecules (79). In this treatment, NMR studies of the binding of halide ions to proteins in solution will be considered.

It is a well known fact that many proteins in aqueous solution bind various small ions, such as the alkali and the halide ions (80). The complexity of the protein molecules has normally prohibited a deeper understanding of the types of interaction of importance. If we for example consider a chloride ion, the following types of binding between protein and anion may be envisaged:

a) (for metalloproteins) complex formation with protein-bound metal ions,
b) ion-pair formation with positively charged groups,
c) binding to a structure-stabilized water lattice near the protein similar to the binding described in refs. 1 and 2.

Studies of ionic nuclear magnetic resonance in protein solutions have partly been performed to elucidate the question of the mode of protein-anion interaction (cf. ref. 5). Most frequently, however, the anion has been used as a "probe" for investigation of certain aspects of protein chemistry such as the function of enzymes.

In the presentation of the quadrupole relaxation method above it was mentioned that the relaxation of a chloride ion bound to a mercury-protein complex is extremely effective. From these considerations it is evident that Hg^{2+}-ions may be used to label proteins for ^{35}Cl NMR studies. This is the basis of the halogen ion probe technique introduced by Stengle and Baldeschwieler (37). In this type of studies the ^{35}Cl NMR line-width is measured in solutions normally containing 10^{-4}-10^{-6} M protein, 10^{-4}-10^{-6} M $HgCl_2$ (or another Hg(II) compound) and 0.2-2 M alkali chloride. In such a system the following types of binding sites for the anions have to be considered:

a) "free" chloride ions,

b) chloride ions in complexes between Hg^{2+} and Cl^-,

c) chloride ions coupled to protein-bound Hg(II),

d) chloride ions coupled to non-metallic protein sites (5).

If the exchange rates for chloride ions are considerably greater than the line-widths, the observed signal will be a weighted average of the line-widths for the different sites (a,b,c and d)

$$\Delta\nu_{obsd} = \sum_i p_i \Delta\nu_i \qquad (5)$$

Here $\Delta\nu_i$ is the line-width characterizing site i and p_i is the probability for a chloride ion to occupy site i. The summation is taken over all sites. At the metal concentrations used in such studies $p_b \Delta\nu_b$ may normally be neglected. (This can also easily be checked by separate measurements in the absence of protein.) $p_d \Delta\nu_d$ is normally smaller than $p_c \Delta\nu_c$, and may be determined by recording the NMR signal in solutions of the metal-free protein (if the metal binding does not cause conformational changes). Of course, the metal-free protein may not be easily accessible, as was the case in the work described in ref. 7.

Since p_a is close to unity and $\Delta\nu_a$ known, a line-width measurement will yield $p_c \Delta\nu_c$. Changes in this parameter on suitable modifications of the solution (e.g. addition of inhibitor, substrate or coenzyme, or changes in pH) give information about e.g. the stoichiometry of the binding of chemical compounds to the protein, and about conformational changes.

Our discussion so far has been concerned with Hg(II) as a "label" and Cl^- as a"probe", and the majority of the investigations have involved this combination (37, 81-87). Recent studies have shown that a large variety of metal labels may be used. Some examples are Cu(II) (6), Co(II) (88), Cd(II) (87) and Zn(II) (6, 7, 24, 87, 89-92). Another probe which has been used is Br^- (^{79}Br and ^{81}Br NMR) (6).

A variety of applications of the halogen ion probe method have been found, and include e.g. the establishment of the existence and nature (i.e. inter- or intrachain) of disulfide bridges (83, 85, 86), the determination of stoichiometry and affinity for the binding of haptens to an antibody (84), the study of the helix-coil transition in synthetic poly-L-glutamate (81), the stoichiometry of the binding of inhibitors to a zinc-containing enzyme, carbonic anhydrase (24), and the binding of surfactants to serum albumin (93).

From the value of $\Delta\nu_i$ for the protein-bound chloride ions observed in a halogen ion probe experiment, the correlation time, τ_c, can be calculated from eq. (3) if the field gradients can be estimated. This leads to correlation times which are orders of magnitude smaller than those expected if the rotation of the whole protein molecule determined the correlation time (94). The reason for this may be internal motion

in the protein molecule (94) or chemical exchange, the rate of which is greater than, or of the same order of magnitude as, the inverse rotational correlation time (95).

A study of aqueous lysozyme (a non-metallic enzyme) solutions illuminated some aspects of the binding denoted d) above (5). Comparison between ^{81}Br and ^{79}Br relaxation shows that the exchange between the protein sites and the bulk solution is rapid compared to the relaxation rates. The observation that the relaxation of ^{85}Rb in aqueous rubidium bromide solutions is unaffected by the presence of lysozyme indicates that the change in microdynamic properties in water on addition of protein is restricted to the near vicinity of the protein molecules. A rather firm attachment of bromide ions to the protein was demonstrated by the observed high energy of activation for the relaxation process.

Titration of a solution of metal-free protein with metal ions, using the halide ion NMR line-width as the parameter of measurement, may provide information concerning the stoichiometry of metal binding and the mode of interaction between protein and metal ion. Thus, it has been observed that the line-width increases with the metal content until it becomes constant when the protein is saturated with respect to metal (83, 87, 88). For E. Coli alkaline phosphatase, an entirely different type of behaviour was observed (6). Thus, the first additions of Zn^{2+} give no increase in either ^{35}Cl or ^{81}Br line-width compared to apophosphatase. Above a molar ratio Zn^{2+} to enzyme of about one, the line-widths start increasing. This shows that the zinc-protein interaction varies with the zinc content. There are several possible explanations of the observed effect. For example the first zinc ions bound may not be accessible for chloride binding, or the residence times of the zinc-bound chloride ions may be

long. Conformational changes and cooperative interactions might also cause the observed effect. The shape of the apophosphatase titration curve with Cu^{2+}, using ^{35}Cl NMR, is similar to that described above for Zn^{2+}. In contrast to the Zn^{2+} - and Cu^{2+} - enzymes, and to Co^{2+}-carbonic anhydrases, no broadening due to the addition of even a large excess of Co^{2+} to an apoalkaline phosphatase solution was detected. This may be explained by the fact that Co^{2+} does not have available a coordination site for chloride. An observation made in this work (6) is that the addition of orthophosphate, which is a competitive inhibitor, to a Zn^{2+} -alkaline phosphatase solution reduces the ^{35}Cl NMR line-width. Comparison of the data for the two anions (Cl^- and Br^-) shows that the relative line-broadening effect of either apophosphatase or metallophosphatases is much greater for $^{35}Cl^-$ than for $^{81}Br^-$. Consequently, the Cl^- ion is a better probe than the Br^- ion.

Another zinc enzyme, horse liver alcohol dehydrogenase (LADH), was also studied by ^{35}Cl NMR (7). In this case, the apoenzyme was not available, and the role of zinc for the chloride binding could thus not be deduced in this way. In contrast to studies on other enzymes, the addition of complexing ligands did not significantly affect the line-width. Titrations with coenzyme (nicotinamide adenine dinucleotide, NADH) were more informative. Since LADH is known to consist of two identical subunits, a stoichiometry of 2 moles of NADH per mole of LADH could be expected. However, it was found that the addition of <u>one</u> mole of NADH per mole of enzyme reduced the ^{35}Cl NMR line-width to a level which is not changed by excess of NADH. The observed stoichiometry indicates that the initial identity of the subunits in the coenzyme-free dimeric enzyme molecule has disappeared upon the addition of one mole of coenzyme. This experiment thus indicates an

interaction between the protein chains. (Similar evidence has been obtained by other methods (23, 96, 97).) If the NADH titration is performed in the presence of an excess of the inhibitor isobutyramide, the stoichiometry of two moles NADH per mole of LADH is observed. A probable explanation for this is that the inhibitor affects the affinity for coenzyme binding, which also agrees with other observations (98). The LADH study (7) shows a new way to investigate interactions between protein chains in oligomeric proteins.

Of course, it is to be expected that, although proteins have received the greatest interest, ion binding to other macromolecules may also be studied by the quadrupole relaxation method. Thus e.g. it was observed that the addition of a humic acid to an aqueous rubidium chloride solution broadened the ^{85}Rb NMR line drastically (99). Consequently, the cation binding to humic acids may be followed by this method (9). A comparison with ^{133}Cs resonance showed that the broadening effect is due to quadrupole relaxation. In contrast to protein solutions no binding of chloride ions was observed (by ^{35}Cl NMR) under the experimental conditions used. The markedly different line-broadening effects of different humus fractions derived from different soils indicate that the method can be used to characterize the humic acids (9). Recent reports have shown that the binding of alkali ions to proteins can also be studied by the quadrupole relaxation method (100, 101).

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